

### **REMARKS**

Claims 1-44 were pending prior to this Response, with claims 3, 5, 31, 32 and 42-44 being withdrawn as subject to a restriction requirement. By the present communication, paragraph [0130] of the Specification has been amended to delete an active hyperlink. In addition, no claims have been added, claims 2-3, 5-8, 10, 17-19, 21, 29-33, 37-39 and 42-44 have been cancelled without prejudice, and claims 1, 9, 11-14, 16, 20, 24, 25, 28 34-36 and 40 have been amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the Specification and original claims. Accordingly, claims 1, 4, 9, 11-16, 20, 22-28, 34-36 and 40-41 are currently pending in this application.

### **The Declaration**

The Examiner asserts that the declaration filed January 31, 2003 is defective because it incorrectly claims priority to a non provisional application under 35 U.S.C. § 119(e) rather than 35 U.S.C. § 120. To overcome the rejection, Applicants submit herewith an unsigned Declaration that correctly claims priority to the application Serial No. 09/771,357 under 35 U.S.C. § 120. Applicants will submit an executed copy of the revised Declaration as soon as signatures of the co-inventors have been obtained.

### **The Objection to the Specification**

The Office Action contains an objection to the Specification for containing an embedded hyperlink and/or other form of browser-executable code. To overcome the objection, by the present communication Applicants have amended the paragraph beginning at line 19 of page 32 to remove the active hyperlink.

In addition, the Examiner has requested that either the drawings of the description of the drawings be amended to set forth the proper sequence identifiers for each sequence. In response to this objection to the Specification, Applicants submit herewith copies of Figures 1-10 with

mark-ups showing proposed amendments to Figures to recite the sequence identifiers for the sequences shown in the Figures. Applicant will provide formal drawings that include corresponding amendments to Figures 1-10 upon allowance of claims in this application.

In view of the amendment and the marked-up copies of Figures 1-10 showing proposed amendments to add proper sequence identifiers, Applicants respectfully request reconsideration and withdrawal of the objection to the Specification.

**The Rejection under 35 U.S.C. § 112, First Paragraph**

Applicants respectfully traverse the rejection of claims 1-2, 4, 6-31 and 34-41 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. Applicants disagree with the Examiner's assertion that the Specification fails to provide sufficient description to enable those of skill in the art to make or use the invention commensurate in scope with the previously presented claims. By the present communication, previously presented claims 2, 5-8, 10, 17-19, 29-31 and 37-39 have been cancelled without prejudice, rendering the rejection moot as to the subject matter of these claims. The rejection will now be discussed with regard to pending claims 1, 4, 9, 11-16, 20, 22-28, 34-36, and 40-41.

The Examiner asserts that the claims do not set forth the relationship between the nucleic acids of a subject and "the same" nucleic acids of a control that is not a comparison of nucleic acids between two individuals. To address the Examiner's concern, Applicants have amended claims 1, 16 and 28 to require detection of a state of hypermethylation in the patient's nucleic acid as compared with "the state of methylation of one or more CpG islands in the promoter of RAR  $\beta$ 2 nucleic acids in comparable samples obtained from normal subjects." Thus, the "control" is not limited to breast tissue of a single normal individual, but is required to be representative of nucleic acids obtained from a plurality of normal, i.e., the absence of

hypermethylation in CpG islands in the promoter of RAR  $\beta$ 2 nucleic acids in a broad population of normal subjects.

Further, the Examiner asserts that there is no teaching or guidance in the specification that hypermethylation in an intron or exon of RARB2 would lead to decreased expression of RARB2 or be associated with breast cancer or any cellular proliferative disorder of the breast, thus causing those of skill in the art to allegedly engage in undue experimentation to practice the invention. However, the invention methods for detecting primary breast cancer, as recited amended claims 1, 16 and 28, require detection of a state of hypermethylation of one or more CpG islands in the promoter of RAR  $\beta$ 2 nucleic acids in the subject's sample as an indication that the subject has primary breast cancer. Thus, hypermethylation in an intron or exon of RAR  $\beta$ 2 is excluded by the claim amendments.

The Examiner acknowledges that the Specification is enabling for an embodiment of the invention described as follows: "a method of detecting primary breast tumors in a subject comprising obtaining nucleic acid from a blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow specimen of a subject and determining the state of methylation of CpG islands of the promoter of RARB2 nucleic acids, wherein hypermethylation of CpG islands in the promoter of RARB2 is indicative of breast cancer in the subject" (Office Action, page 3). To reduce the issues and expedite prosecution, Applicants have amended independent claims 1, 16 and 28 to focus the invention on the subject matter that the Examiner has indicated is allowable. However, Applicants specifically reserve the right to pursue other embodiments of the invention in a subsequently filed application.

In view of the amendments and for the reasons discussed above, Applicants submit that the Examiner's concern that those of skill in the art would have to engage in undue experimentation in order to practice the claimed invention has been overcome. Accordingly,

reconsideration and withdrawal of the rejection of claims under 35 U.S.C. § 112, first paragraph, are respectfully requested.

**The Rejection under 35 U.S.C. § 102(a)**

A. Applicants respectfully traverse the rejection of claims 1, 4, 28-30, 34, and 37 under 35 U.S.C. § 102(a) as allegedly being anticipated by Ferguson et al. (*PNAS* 97:6049-6054, 2000; hereinafter "Ferguson"). By the present communication, previously presented claims 29, 30 and 37 have been cancelled without prejudice, rendering the rejection moot as to the subject matter of these claims. The rejection will now be discussed with regard to pending claims 1, 4, 28 and 34. Applicants submit that the invention methods for detecting primary breast cancer in a subject, as defined by amended claims 1, 28, distinguish over the disclosure of Ferguson by requiring:

determining the state of methylation of one or more CpG islands in the promoter of RAR  $\beta$ 2 nucleic acids isolated from a sample comprising blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow, or a combination thereof of the subject, wherein a state of hypermethylation of one or more CpG islands in the promoter of RAR  $\beta$ 2 nucleic acids as compared with the state of methylation of one or more CpG islands in the promoter of RAR  $\beta$ 2 nucleic acids in comparable samples obtained from normal subjects is indicative of primary breast cancer in the subject.

By contrast, Ferguson is absolutely silent regarding all elements of the invention methods for detecting primary breast cancer as defined by amended claim 1 and 28. The Examiner asserts that Ferguson discloses that hypermethylation of the sigma promoter, for example, a CpG rich region of the 14.3.3 sigma gene promoter, is largely responsible for silencing of the sigma gene and occurs in a majority breast cancers. However, Applicants respectfully submit that Ferguson fails to disclose that hypermethylation of CpG islands in the promoter of the RAR  $\beta$ 2 nucleic acids, as compared with the methylation of comparable nucleic acids in normal samples, is indicative of primary breast cancer.

As Ferguson fails to disclose each and every element of claims 1, 4, 28 and 34, as would be required to establish anticipation under 35 U.S.C. § 102(a), Applicants respectfully request reconsideration and withdrawal of the rejection over Ferguson.

B. Applicants respectfully traverse the rejection of claims 1-2, 4, 6-31 and 34-41 under 35 U.S.C. 102(a) as allegedly being anticipated by Sirchia et al. (*Oncogene* 19:1556-1563; hereinafter "Sirchia"). Applicants enclose with this Response a Declaration under 35 U.S.C. § 131 stating that the co-inventors of the present application, and of the prior application on which it relies, conceived and reduced the invention methods to practice in the United States prior to the date of publication of the Sirchia article. Moreover, the Declaration states that Nicoletta Sacchi, who is named as one of the co-authors of the Sirchia et al. reference, is one of the co-inventors of the present application and contributed to conception of the invention described in the priority application. By contrast, Silvia M. Sirchia, Anne T. Ferguson, Elena Sironi, Smitha Subramanyan and Rosaria Orlandi, who are named as co-authors of the Sirchia article, contributed to the research effort that led to the Sirchia reference, but did not contribute to conception of the invention as described in the present application or as described in the priority application.

Thus, the disclosure of Sirchia that is relied upon in the rejection of claims 1-2, 4, 6-31 and 34-41 of the present application for alleged anticipation was conceived and reduced to practice by the co-inventors of the present application prior to the date of publication of the Sirchia reference. Accordingly, Sirchia is not available as prior art under 35 U.S.C. 102(a) and reconsideration and withdrawal of the rejection are respectfully requested.

C. Applicants respectfully traverse the rejection of claims 1, 4, 28-30, 34, 37 and 38 under 35 U.S.C. § 102(a) as allegedly being anticipated by Esteller et al. (*Cancer Research*, 58:4515-4518; hereinafter "Esteller"). By the present communication, previously presented claims 29, 30, 37 and 38 have been cancelled without prejudice, rendering the rejection moot as to the

subject matter of these claims. The rejection will now be discussed with regard to pending claims 1, 4, 28 and 34.

Applicants submit that the invention methods for detecting primary breast cancer in a subject, as defined by amended claims 1, 28, distinguish over the disclosure of Esteller by requiring:

determining the state of methylation of one or more CpG islands in the promoter of RAR  $\beta$ 2 nucleic acids isolated from a sample comprising blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes, bone marrow, or a combination thereof of the subject, wherein a state of hypermethylation of one or more CpG islands in the promoter of RAR  $\beta$ 2 nucleic acids as compared with the state of methylation of one or more CpG islands in the promoter of RAR  $\beta$ 2 nucleic acids in comparable samples obtained from normal subjects is indicative of primary breast cancer in the subject.

By contrast, Esteller is absolutely silent regarding all elements of the invention methods for detecting primary breast cancer as defined by amended claims 1 and 28. The Examiner asserts that Esteller discloses a method for determining the methylation state of CpG rich region of the GSTP1 gene promoter using methylation specific PCR in samples of breast tumor from a subject and compared to the methylation status of the nucleic acids in normal breast tissue.

However, Applicants respectfully submit that Esteller fails to disclose a method for determining the presence of primary breast cancer in a subject by determining the presence of hypermethylation of CpG islands in the promoter of the RAR  $\beta$ 2 nucleic acids, as compared with the methylation of comparable nucleic acids in normal samples, as is required in the invention methods.

In re Application of:  
Sukumar et al.  
Application No.: 10/059,579  
Filed: January 28, 2002  
Page 15

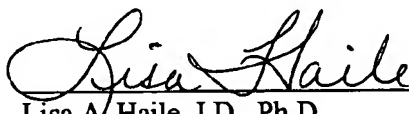
PATENT  
Attorney Docket No.: JHU1630-1

Therefore, Esteller fails to disclose each and every element of claims 1, 4, 28 and 34, as would be required to establish anticipation under 35 U.S.C. § 102(a). Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection over Esteller.

In view of the above amendments and remarks, Applicants submit that all rejections of the claims are overcome and Applicants request favorable action on all pending claims. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures: Unsigned Declaration of Inventors  
Marked-up Copies of Figures (18 sheets)  
Declaration of Applicants Under 37 C.F.R. §1.131



(SEQ ID NO:105)

Cyclin D2 promoter, MSP primers  
Accn. No. U47284 Promoter region analyzed: -1616 to -1394 bp

1 gagctCGagc caCGcagtc cGctgcaCG tgcagcttg CGcagcacat cagggCGctg  
61 gtctctccc ttctctctgg agtgaatac accaaaggCG GCGgtggggg tgggggtgga  
121 CGggaggaaag gaggtgaaga aCGccacca gatCGtatct cctgtaaaga cagccttgac  
181 tcaaggatCG Gttagag ~~gaggtgaaga~~ CGacCGctgc ~~gaggtgaaga~~ gCGgacttc aCGcagtcCG  
241 gctcccaggg agaaagcctg gcagagtga gCGGaaacC GgagggtCGG CGaggatgCG  
301 ggCGaaggac CGagCGtgga ggcctcatgc ctcCGgggaa aggaagggtt ggtggtgttt  
361 gCGcaggggg agCGaggggg agCGGgacct aatccctcac tCGccccc cccctccCGg  
421 gccatttctt agaaagctgc atCGgtgtgg ccaCGgag ~~gaggtgaaga~~ tCGggCGgct  
481 tgtcagcaga tgcagggggCG aggaagCGgg ttttctctgC GtggcCGctg ggCGgggaa  
541 cCGctgggag ccttgccccC GgcctgCGgC GgccttagaC GctgcacCGC GtCGccccac  
601 ggccccCGaa gagccccag aaacaCGatg gttctgctC Gaggatcaca ttctatccct  
661 ccagagaagc acccccttc ctctctaata cccacctct cctccctctt ctctctctgc  
721 acacactctg cagggggggg cagaagggaC Gttgttctgg tccctttaat CGgggctttc  
781 gaaacagctt CGaagttatc aggaacacag acttcaggga catgaccttt atctctgggt  
841 atgCGagggt gctattttct aaaatcaccc cctcccttat tttcaccta agggacctat  
901 ttctaaattg tctgaggtca ccccatcttc agataatcta cctacattc ctggatctta  
961 aatacaaggg caggaggatt aggatcCGtt ttgaagaagc caaagtggga ggtCGtatt  
1021 ttggCGtgct acacctacag aatgagtga attagaggc agaaatagga gtCGgtagtt  
1081 ttttgtgggt tgcctgtcCG gggcccttgg catgcaggct ggatggagg agaggggtgg  
1141 ggggtggCGg gggacCGCGt ttgaagtggg gtCGggccag ctgctgttct ccttaataac  
1201 gagaggggaa aaggaggga gaggaggag attgaaagga ggaggggagg accCGggaggg  
1261 gaggaaggg gaggaggaac cagagCGggg aggCGCGggg agaggaggga gagtaactg  
1321 ccagccagc ttgCGtcacC GcttcagagC GgagaagagC Gaggaggga gagCGagacc  
1381 agttttaagg ggaggacCGg tgCGagtga gacccccCGa ggtctgtctC Gccaccacc  
1441 caatcctCGc ctcccttctg ctccaccttc tctctctgcc ctacacctc cccCGaaac  
1501 cccctattta gccaaaggaa ggaggtcagg gaaCGctct cccctccct tccaaaaaac  
1561 aaaaacagaa aaacctttt ccaggcCGgg gaaagcagga gggagagggg cGcCGggct  
1621 ggcc gag

FIGURE 1A





MSP Unmethylated 223 BP

GT TATGTTAAGT TTGTTGTAAG

Forward UM 22 BP MT 56

(SEQ ID NO: 21)  
(SEQ ID NO: 22)

T AAATCCACC AACACAATCA

Reverse UM 21 BP MT 56

MSP Methylated 223 BP

TACGCTGCTTAAAGTTCGATGCG

F M 19 BP MT 58

(SEQ ID NO: 23)

CGAATATATATGCGTGAATACG

R M 20 BP MT 56

(SEQ ID NO: 24)

MSP External primers 287 BP

TATTT TTTGTAAAGA TAGTTTGTAT

EXT.F

(SEQ ID NO: 129)  
(SEQ ID NO: 130)

TACAACCTTCTAAATAAATACCC

EXT.R

FIGURE 1B



(SEQ ID NO:106)

Twist Promoter: Accn No. AC003986

Promoter Region analyzed: nts -51145 TO -51750

1 cattggactg ggtttctctc cacCGaagag tgaactctg cctctttCGa gcaacttcCG  
61 agCGtagtc ctttgatgt tggggagCGt cagactgggt CGttgtagag gggaaaggag  
121 gggccagaag ggCGagagag caggCGgga CGcaaatcct cagccccCGC GgCGGccac  
181 Gtcttcagaa aCGccaggac ctCGggctg ggcCGcCGG gttggcctt tggaaactcaa  
241 ggttCGtct acctgacct tgggtggctc CGCGgttgac actttctttg gcatgcccc  
301 ccacccCGCG ccacacacc cccccagcc cagcaatcca aatCGgcccc aCGgacctag  
361 agggctcttg ggCGagatga gacatcccc actgtgtaga agtgttgcc attgctgtg  
421 tcacagcca CGgattgg gctgccaCG tggccaggac agtctcttc GacCGcttcc  
481 tgggctgCGc taggttCGg gggCGctgc CGcaCGctc GgCGgggaag gaaatCGccc  
541 CGGccCGcC GagggaaggC GaCGgggag gaaaggagg ggCGctagg aggCGggtgg  
601 aggggCGgc CGccCGggc aggtCGctt tgaatggtt gggaggCGa attgttagac  
661 ccGaggaa gagggtggga CGggggagg ggaactggaaa gCGgaaactt tccataaaa  
721 cttCGaaaag tccctctcc tcaCGtcagg ccaatgacac tctgcccc aaactttCG  
781 cctgcaCGga ggtataag cctccaagtc tgcagctctC GccCGcttc cagacacctc  
841 gCGggtctg cagcacCGc accGtttcca ggaggcctg CGgggtgtgC GtccagCGt  
901 tggCGcttt ctttttggga cctCGgggc atccacacCG tccccctccc ctccCGctc  
961 cctccCGcc tccccCGcC GcctccCG CGgaggtccc tccGtcCGt cctcctgctc  
1021 tctctcCGG gggCGcatC GccCGggcCG gCGcCGcC Ggggggaagc tggCGggctg  
1081 agCGccCG cttctctct ctgcccCGg ccCGGaggc caCGGtCGc CGctCGagag  
1141 atgcagg aCGtgtccag ctCGccagtc tCGCGgCG aCGacagcct gagcaacagc  
1201 gaggaagagc cagacCGga gacCGcCG agCGgcaagC GCGgggaCG caagCGGCG  
1261 aCGagcaggC GcaCGgCGg CGgCGgCGg gggccCGg gagCGgttgg 999CGtCGga  
1321 ggCGGCGaCG agcCGggcag cCGgccccag ggcaagCGG gcaagaagtc tGCGggctgt  
1381 ggCGGCGgCG gCGGCGGg CGgCGgCGc Ggcagcagca CGGCGGCGg gagtCGGcag  
1441 tcttaCGagg agctgcagc GcagCGggtc atggccaaCG tGCGggagCG ccagCGcacc  
1501 cagtCGctga aCGaggCGt CGcCGGctg CGgaagatca tccccCGct gcccCGgac  
  
1561 aagctgagca agattcagac cctcaagctg GCGgccaggt acatCGactt cctctaccag  
1621 gtctccaga GCGaCGagct ggactccaag atggcaagct gcagctatgt ggtcaCGag  
1681 CGgtcagct aCGcttctC ggtctggagg atggagggg cctggtccat gtcCGGtcc  
1741 cac cagg CGgagcccc caccctccta gcagggCGg agaccCGgt aaggacCGG

FIGURE 2A -- FIGURE 2B



Unmethylated 193 BP  
tt TGGatggggt tggatTGT FUM (3) 21 BP AT 58 (SEQ ID NO:109)  
c ctaaccCAaa CAacCAacc RUM (3) 20 BP AT 60 (SEQ ID NO:110)

Methylated 200 BP  
FM (5) 20 BP AT 58 (SEQ ID NO:107)  
RM (4) 19 BP AT 58 (SEQ ID NO:108)

External primers 371 BP  
Gagatgagatattattttattgtg EXT F (SEQ ID NO:131)  
aacaacaatatcattaacctaac EXT R (SEQ ID NO:132)

FIGURE 2C



RAR beta promoter, MSP primers

ACCN NO. AF157483

(SEQ ID NO: 91)

Promoter region analyzed: nt -196 to nt -357

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1  gtgacagaag tagtaggaag tgagctgttc agaggcagga gggtctattc ttgcccagaag
61  gggggaccag aattcccat gGagctgtt tgaggactgg gatgcGaga aCGGgCGGg
121  gGgGgGgagg gtttgtctgg gcacCGtCGg ggtaggatcC GgaacGcatt CGgaaggctt
181  ttgcaagca tttacttggg aggaagaatt gggatctttc tgggaacccc CCGGgCGGg
241  tggattgGC Gagcaagcct gaaaaatgca attgaacac agagcaccag ctctgaggaa
301  ctCGtcccaa gccccccatc tccacttct cccctCGag tgtacaaacc ctgcttCGtc
361  tgccaggaca aatcatcagg gtaccactat ggggtcagCG cctgtgaggg atgtaagggc
421  tttttCGca gaagtattca gaagaat [REDACTED] atttacactt gtcacCGaga taagaactgt
481  gttattaata agtcaccag gaatCGatgc caatactgtC Gactccagaa gtgctttgaa
541  gtgggaatgt ccaagaatc tgtcaggaat gacaggaaca agaaaaagaa ggagacttCG
601  aagcaagaat gcacagagag ctatgaaatg acagctgagt tggacGatct cacagagaag
661  atcCGaaaaa gtcaccagga aactttccct tcactctgcc agctgggtaa atacaccaCG
721  aattccagtg ctgaccatCG agtcCGactg gacctgggcc tctgggacaa attcagtgaa
781  ctgggccacca agtgcattat taagatCGtg gagtgtgcta aCGtctgcc tggtttcaat
841  ggcttgacca tCGcagacca aattaccctg ctgaaggcCG cctgcctgga catectgatt
901  cttagaattt gcaccaggta taccocagaa caagacacca tgactttctc agaCGgcctt
961  accctaaatC Gaactcagat gcacaatgct ggatttggtc ctctgactga ccttgtgttc
1021  acccttgcca accagctcct gcctttggaa atggatgaca cagaaacagg ccttctcagt
1081  gccatctgct taatctgtgg agacCGccag gaccttgagg aacCGacaaa agtagataag
1141  ctacaagaac cattgtctga agcactaaaa atttatatca gaaaaagaCG acccagcaag
1201  cctcacatgt ttccaaagat cttaatgaaa atcacagatc tCGtagcat cagtgtctaaa
1261  ggtgcagagC Gtgaatttac ctgaaaaatg gaaattcctg gatcaatgcc accctcatt
1321  caagaaatgc tggagaattc tgaaggacat gaaccttga ccccaagttc aagtgggaac
1381  acagcagagc acagtcctag catctcacc agctcagtg aaacacagtg ggtcagtcag
1441  tcaccactCG tgcaataaga ca
```

FIGURE 3A



Unmethylated 163 BP  
ggattgg gatgtTGaga atGT FUM 21 BP AT 60 (SEQ ID NO: 92)  
C Aaccaatcca accAaaacAA RUM 21 BP AT 60 (SEQ ID NO: 93)  
Methylated 163 BP  
ggattgg gatgtTGaga atGT FM(2) 19 BP AT 60 (SEQ ID NO: 135)  
C Aaccaatcca accAaaacAA RM(2) 19 BP AT 58 (SEQ ID NO: 136)  
External primers 266 BP  
gtaggagggttttattt ttgtt EXT (2) F (SEQ ID NO: 133)  
aattacattttccaaacttactc EXT 4 (2) (SEQ ID NO: 134)

FIGURE 3B





UnMethylated 213 BP

tTggtTgG aagttgggTG FUM 18 BP AT 56 (SEQ ID NO: 71)

gtaTgtg attTGaagtT\_gtatt (SEQ ID NO: 98)

aatac aacttcaaat ca~~ca~~tac

**Metaphors**

FM 18 BP AT 58 (SEG ID AD: 69)

taCGtg attCGaagtc Gstat (SEQ ID NO: 99)

ATTACCGAATTCGATGAGGACCGGAG RM 20 BP AT 56 (SEQ ID NO:70)

**FIGURE 5C**

Sequencing 307 BP

atcttggtatcaatgggtgtgtaac Hox A5 Seq. F 23 BP AT 56 (SEQ ID NO: 73)

ggag ggaattaagt atatgtt (SEQ ID NO: 100)

aaacatataccttaattcccctcc Hox A5 Seq. R 21 BP AT 56 (SEQ ID NO: 74)

ccaggtgta cagccagccg gc (SEQ ID NO: 75)

Hox Exp F 20 BP AT 60

Hox Exp R 18 BP AT 62

Hox Exp F 20 BP AT 60 (SEQ ID NO: 76)

FIGURE 5D







SEQ ID NO: 102 (CONT')

1621 caggattttg ccattctctg cacagcctga gggagactaa caggcctctt tgcagagggg  
1681 tagctggtaa gaccgtttct tccctgtcgg ccagcactgc ccgctcccc cccacaccca  
1741 tctcattctc atgcgctgcc tgcaccaacc catggagccc gtccatctgt ctggtgtgtg  
1801 gtgcggtgtg tgtgctggtg gtggtagggt ctccaggagc tccccgctaa gcagaaggat  
1861 cgggatatag gcaaggcta aagccccagc cccattgtgg actgagggaag tacgttcgag  
1921 cagagcagct ctccagctgg aagaggaggt ggagggtgag gctggggaga gcatggcgaa  
1981 cctgccctga ggtgcttggg tctgtgctgg tggggtcctg gtatgcaggg gccaccggtc  
2041 actaacactc ttatgtcctg gctttctgtc cccgctgagc ttctctcac ccgcccgttt  
2101 tctctcctgc ttcatgtcct gctgcctaag ccttggccct tctctcggc agaggcaggt  
2161 gctgtggcag caccctctcc caccaccggg cccctgcagg ccgctcccc cctcccaggc  
2221 ctgctaaccc tctctctct ccttctttgc tgtcctgccc gggatctcca gctgtgcggg  
2281 gggcttaagg acctcctgag gaccgtgct ctctgctct ccaggaaatgg cctgggggga  
2341 gccaggcacc cggcactctc acctgcctaa cctgtgccc atctggcacc atctgtgctt  
2401 acagggtctg cccccagcc tggccggcct gtgtgctctc taggacccca tagggggcag  
2461 gggctggcct ctttgcccca ttcccgctcc atgccggcca ggtgtgtaga agccataacg  
2521 cagcagacca tcagcacaat aatgtgactc tacgtgata tctctccct ctcctccact  
2581 gacttcccc tcccggattt gtgaggtgtc aagactagga atctggcctt agagcctgcc  
2641 cctccacccc ctacagatcag gcatagccat agtcaagccc agcaggtttc ctcaggaggt  
2701 gctcggggtg ttgatggtgg atgacgtgc tgaacaagt ttgtgactgt tctaagcaca  
2761 actggcttga tactgttccc acggcctgtc cactctccc ccccaacct ccaccagagt  
2821 aggtaggatg tagggagggt gcgtgcgcc ttgtctctag gcactgaggg accaagctag  
2881 ccgtgcacag cccatacac ttcaggggcg taaaggaaag agctgagcca aggaaaaatca  
2941 gctgagccca gggctggggg ctgcttgtct gctatcctgt acctttttt ttttaacca  
3001 aaataaagat tcccccttc ttgccatacc attgctgtc tgggtggcgc ttactttgg  
3061 gggccaggga tgggacctgc agtgggctg tggaaacatat ggtccccct cgctccagc  
3121 ttcttccag ctggccagtg ctgctctgga gattacaag cacaacgaag ccaggagga  
3181 cacaggaaaa gtggctgaca tcttttccac tctgccccct cagaactctt ggtctcaatt  
3241 ccagacacca ccagcctta gctgacctct ggtattctgat aggtccaggt gcagggtgag  
3301 acagagggtt taactccagt ttgggactgc catacccatg aactgagccc agcccagggt  
3361 aacgatctca tggaaacttc tctctccca gttgctgcac tacatcaaga tacacacatg  
3421 tgcatacact gtactatggg ctaaaaaaat acgtaccgtt accgttcagc aagggtctgc

FIGURE 6B



(SEQ ID NO: 102 CONT)

3481 cgagtcccg gccatttc tcatttaac ctgtgaggag gatgatgtca gcccttttac  
3541 agatgaggga actgagactc aaggaagaaa caggagctgc ccaaggctac ccagctggca  
3601 aagcagcaaa tcccagatcg gaacctgac tctgccccg gctctgagcc atctgcacta  
3661 cccaagggaat gaatacagcg gtgggaggat gagatcttgg agaacccta aaattagaga  
3721 atgtcatagc cagtagaggg cttagagttg atctgggcca gccctcttgt tttactgatg  
3781 gagaaattga agcccagagg caggaaggga cctgcccagg gccattataac agagctggga  
3841 tgcagtccta cactctgacc tcattccatt ctctctccat aaattctgca ctgtctctag  
3901 actggactgg tttagatgtg ggatactcta aacagcagtg ccttcaagag aaaaagaatc  
3961 agaactacga atcactaaa agtaatgtaa gctactctgg gcacactgcc tatggggtcg  
4021 ccctgtctca caaggagcca caaaaataat taaaataatt taatatccct tcccaagggt  
4081 aaccagtaaa gtaagctctt ggctaggtaa ctggactctt gttcacact agccagtggg  
4141 aaaagggtgct agagcttctt ctggccacct gtttaatttg atcattccaa gacagaaca  
4201 tttcttagga agtcttcttct agaattctacc tgggtgtccct cccactgcta tcagagccct  
4261 gtccctctgc ctacgtggag gttagagaca aatggttgc gctttcttca tcacaacct  
4321 tcaaaacctt ttattaccag ctaagaagga ttggttgact atgggccaga gccctgagc  
4381 ctgctggtag aatggatgct gtacaggagg gtggggagg agcaggcaga atgaggaaaag  
4441 cccctttgag ctgcaacccc agctcctgtc ctgctgactc agacagctga ctgtggagct  
4501 ccattgccctg ccagggccctg ctgcctctcg cccgtctgag ctctgaact tgggaaatgg  
4561 agggccagag gcaaaaggag gtacctgaga caggaactga gtcaggatca acaggccaga  
4621 gcgggcagga ggtatcaggc agcctggctc ccagatgcac cctgagctc cagcagggga  
4681 ggagtaggaa tgaaggggct tcttgccct tgctcatggc tatgctggag gcgtgaacca  
4741 ccaccaggct ctctggctta agtggcgagg agcaaatggc cctccctgg actcaggctc  
4801 caaagtctct gggcctgcct tccaggttcc cagtgtctg ggtctccag ctttccccag  
4861 gacttgggga agcccggct ggatgactag tacaatatga gggccctgag gttccaggac  
4921 ctgctgaggt cacaggaata tcctagatca agcttgcca acccacggc cacaggctgc  
4981 atgtggccca gaatggcttt gaatgcagcc caacacaaat tagtaacct tcttaaaaa  
5041 ttatgagatt tttttgcaa ttttttttt ttttttagct catcagttat tggtagtgtt  
5101 ggtatatatt atgtgtggcc caagacaatt ctccaatgt ggcccaggga agccaaaaga  
5161 ttggacacgc ctgtcctaga tggagaggaa ggaggcagtg ctgagcacat ctggccattc

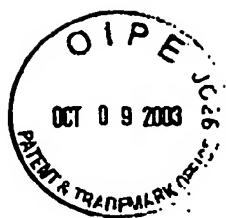
FIGURE 6C



(SEQ ID NO: 102 cont.)

5221 atccatctgg agagagaagg ctatgggcaa actgtctcct ctccccgtga gacaccagc  
5281 tgggaaggct tggcctttgg taagtcctgg ctctgggtcc ttctcattt cacagaaact  
5341 aactctatgt tagtgctttg tgagtatatg ttgatacaaa taaagttgac gggatttttt  
5401 cacatgataa taatagttgt catctggccg ggcattgtgg ctatgccta taatttcagc  
5461 actttggaag gctgaggcag gtggatcact tgaggtcagc tgttcgagac cagcctggcc  
5521 aacatgggta aaccacatct ctacttaaaa aaaaaaaaaa taaaaaatt agctgggtgt  
5581 ggtggtgcac ccttgtaac ccagctactc gggaggctga ggcaggagaa tcaattgaac  
5641 ccaggagggt gaggttcag tgagctgaga ttgtgccact acactccagc ctgggtgaca  
5701 agagcgaac tccgtctcaa aaaaaagaa aataataata ataatagttg ccatccattc  
5761 tactgtgctt tccattaact cgttaaatcc tcacaagtcc cattttatag ttacaggaac  
5821 tgagggtcac agagcttaaa tcaattggcc aaggccacaa acagctataa gaattacatt  
5881 taggcagtct gattccaaa atactagtct attctgtatc tcatagacaa acaatacata  
5941 ttcacttttt tgtgtttgtt ttgttttgag acggagtctt gctctgtcac ccaggctgga  
6001 gtgcagtggc gccatctcgg ctcatgcaa cgtccgctc ccgggttcaa gcgattctcc  
6061 tgcctcagcc tcccgagttag ctgggactac aggcattgac caccatgccc ggttaatttt  
6121 ttgtattttt agtagagaca ggttttctct gggttagcca gaatggtctc gatctcctga  
6181 ccttgtgatc caccacctc agcctccaa agtgcctaga tgacaggcgt gagccaccgc  
6241 gtccgacctt tattcactat ttataaattg gagagaataa gaaatcaaa agggccagggt  
6301 gtgtgactc acacctgtaa tcccagcact ttgggaagcc aaggcaggag gattgcttga  
6361 acccagaagt tcgagaccag cctgggcaac atggtgagac cctgtctcta caaaaaatac  
6421 aaaaattagc tgggcgttgt ggtgagcacc ttattcttag gaagctgagg caggaggatc  
6481 acctgaggcc aaggagggtt agactgcagt gagctgtgat cataccactg tacttcagcc  
6541 tggacatcag agtaagacc tctctaaa aggaaattg agaagaaaga aaatcaaaagg  
6601 gaagcaaat cactcactct cactactca agataccctc tagaagtgg tattttagtg  
6661 tggttcctat tgttttctgt gtcagtctc tgatttgagc aaaatctttg gfacgtcaaa  
6721 cttaaaaacc ccttacttc cttgaaacc ctgtagcatt agcccagaca tgtccctact  
6781 cctccttggt gcaaaagaaa ggtatctctc ttgtgtccc agagtcttg ctaagcctc  
6841 cctccaggag ggaagatgag tgttcagaca ctgagagttag ctgggggaga cacaggcctg  
6901 tgaattatc ctggctcaac tattaggtcg gcagaatccc agtgaaggga gcctacctc  
6961 tgagcccat ctaagctttg gctatgggtg ggcagataaa gcaggaaatcc atccctatag

FIGURE 6D



(SEQ ID NO: 102 cont.)

7021 gctcaatgcc aacaccctta ggtgaaactc ttgatgaaac ttgaggccag ggtcccgga  
7081 agcaggaaaa gaacgttggc aacagaggtc tccatctctg aggactctgc cagggggtcag  
7141 agatggggca atggtcaaaa ggaaggaaaca ggccaggcac agtgggtcat gcccataatc  
7201 ccagcacctt gggaggctga ggcaggagga tcgcttgagc ccaggagttt gagacctgcc  
7261 tgggcaatgt agtgagatct gctctctatt taaaaaaa aaaaaggaaa gaacaaagtaa  
7321 acttctgaga aacaggctgg gggaggcatc acgtagctgg aattgctgcc ccataaaaca  
7381 gaatggtatg tgtactgcc accctccctt ctgagtcctc tctctcccca ggttgctagc  
7441 gtccccctgg gggatcaaac tggactgctt cccagcctca gcagagagc agtctgagtc  
7501 aggcaggaaa gtgggacagc cggggagctg gaccccaccc tctgtgagcc ccgctggtac  
7561 ctgatggcat gtggcttggg gagggcaggt gacctggcgt gaggggccag agggtaaatc  
7621 ctcaaacaaag tggcaacagg ccaccaactt gaaagggaaa atgtgtagat gatgggaaat  
7681 gtgtccaaca aacctactgg gtgactaatt acaaaggctg ggttgagagct tcagaggctg  
7741 ctgtttaaac acttcattaa gcggcacctc gaaagctgcc acctgcgcat tctgggagct  
7801 cagaggggac cctgaggggg aatgaggcct ggaggatgga accatcttca ggtagactga  
7861 gaaggagcct ggatctcact tccaaacaca gtctggagct cataggtcag aggcctcaat  
7921 gggagaaaaag ctaaaaggag aggtgtcaga aaggagtctc aggaattgg tggctatgtg  
7981 actttgagca aatctcacc ctctctgaga cttagtgttc ccactctat ggtcctgtgt  
8041 gtgtcacaga gacatgggtg ggattaaatt cgatcgtgat atgaaagtgc ttgggaaact  
8101 ccatggccct acctaaacat gagttatcct cactgaacc aaggggggaa gttacctggc  
8161 aggattagga acccatcct cctgaacctt tatgggctct gtcgaggctg aagcagccag  
8221 gggctaaagc cagtccttag cccctggag ggcactgtga aagtggatct gatttgagaa  
8281 gccgtttcct gatgtggca gccatgtgat gccagccccg acaagaggg ggagccctgg  
8341 agcctggaaa ggtgccagt gagggtgggc ccagcccgag atttctctg ctgactgttc  
8401 tgatgattca cccccacat ccagcctttt tacctttact gcagagccgg aaagggtgtg  
8461 gggagagag gagaggagg caggtcttgg gccctgggcc cgccccctgc tctccccac  
8521 ccttctctgg gcctggccac ccagccaaa ggcaggccaa gacagagga gacacagat  
8581 ccggcattgg tcccaggcag cagttagccc gccgcccgcc tgtgtgtccc cagagccatg  
8641 gagagagcca gtctgatcca gaaggccaa gtcggcagag agcccgaaag ctatgagac  
8701 atggcagcct tcatgaaagg cgcctgtgag aagggcgagg agctctcctg cgaagagcga

FIGURE 6E



SEQ ID NO: 102 (CONT)

8761 aacctgtctt cagtagccta taagaacgtg gtggcgccg agaggctgc ctggaggggtg  
8821 ctgtccagta ttgagcagaa aagcaacgag gagggctcgg aggagaagg gcccaggggtg  
8881 cgtgagtacc gggagaaagt ggagactgag ctccaggggc tgtcgacac cgtgctgggc  
8941 ctgttgga gcaacctcat caaggagcc ggggacggc agagcgggt ctctacctg  
9001 aagatgaagg gtgactacta ccgtacctg gccgaggtg ccaccgtga cgacaagaag  
9061 cgcatactg actcagcccg gtcagcctac caggaggcca tggacatcag caagaaggag  
9121 atgccgcccc ccaaccccat ccgctgggc ctggccctga actttccgt ctccactac  
9181 gagatcgcca acagccccga ggaggccat tctctggcca agaccattt cgacgaggc  
9241 atggtgac tgcaacacct cagcaggac tctacaaag acgacacct catcatgcag  
9301 ctgtgcgag acaacctgac actgtggag gccgacaacg ccggggaaga ggggggcgag  
9361 gctcccagg agccccagg ctgagtgtg ccgcccaccg cccgcccctg cccctccag  
9421 tccccaccc tgcgagagg actagtatg ggtggaggc cccaccttc tccctaggc  
9481 gctgttctt ctccaaagg ctccgtggag agggactggc agagctgagg ccacctggg  
9541 ctggggatcc cactcttct gcagctgtg agcgaccta accactggc atgccccac  
9601 cctgtcttc cgcaccgct tctcccgc ccaggacca ggtacttct cccctcctt  
9661 tgcctccctc ctgcccctg tgcctctgat cgtaggaaat gaggagtgc cgccttgtg  
9721 gctgagaact ggacagtgg agggctgga gatgggtgtg tgtgtgtgtg tgtgtgtgtg  
9781 tgtgtgcg cgccagtg caagaccgag actgagggaa agcatgtctg ctgggtgtga  
9841 ccatgttcc tctcaataa gttcccctg gacctcctc ctgtctctt tccagtctt  
9901 ggcgatggc tgggagtgg actggaatc gacttagaga cctgaattt ggacctctga  
9961 gttaggggcc tgaactcct aggtggctc gtggcccgca cgcaagactt tgagtccagg  
10021 tgaggccggg gtcc

FIGURE 6F



(SEQ ID NO: 103 CONT)

1441 gggtaaggag ttcaaggcag cgccacacc cgggggctct ccgcaaccg accgctgtc  
1501 cgctccccc cttccgccc tccctcccac ctactcattc accaccacac ccaccagag  
1561 ccgggaaggc agcccaggc cccgggcccc cccgtctcct cgcgcgacac ctggacttcc  
1621 tcttgctgca ggaccggct tccacgtgtg tcccggagcc ggcgtctcag cacacgctcc  
1681 gctccgggcc tgggtgccta cagcagccag agcagcagg agtccgggac ccgggcggca  
1741 tctgggccc gtaggcgc gccgaggcca gcgctgaacg tctccaggcc cggaggagcc  
1801 gggggggcgc cgggtctgag cctcagcaaa tgggctccga cgtgcgggac ctgaacgcgc  
1861 tgctgcccgc cgtcccctcc ctgggtggcg gggcggtctg tgccctgctt gtgagcggcg  
1921 cggcgagtg ggcgcggtg ctggactttg cgcgccggg cgtcteggt tacgggtcgt  
1981 tgggcggccc cgcgcgcga cgggtccgc cgcaccccc gccgcgcgc cctcactcct  
2041 tcatcaaca ggagccgagc tgggcccggc cggagccgca cggaggagcag tgcctgagcg  
2101 ccttactgt ccactttcc ggccagtcca ctggcacagc cggagcctgt cgctacgggc  
2161 ccttcgggtcc tctccgccc agccaggcgt catccggcca ggcagagatg ttctctaacg  
2221 cgcctacct gccagctgc ctgagagcc agccgctat tcgcaatcag ggtaagtagg  
2281 ccggggagcg cccta

FIGURE 7B



SEQ ID NO: 104 CONT

1681 ccactatcct tgtgggtgga ccaggagtCG gttCGagggt gctccactt agaggtcaCG  
1741 CGCGGCGtCG ggCGtctctg agacCGtCG gctccctggc tCGtcaCGt gggtctcaggc  
1801 actactcccc tctacctcc tctCGgtctt taaaagggaag aaggggctta tCGttaagtC  
1861 Gcttgtgac ttttcagttt ctccagctgc tggctttttg gacacccact ccccCGccag  
1921 gaggcagttg caagCGCGga ggtgCGaga aataactgcc tcttgaaact tgcagggCGa  
1981 ~~gaggcagttg caagCGCGga ggtgCGaga aataactgcc tcttgaaact tgcagggCGa~~  
2041 ctgCGgggca gggctggCGc cCGgagcctg agctgcagg ggtgCGtCG ggttctggtg  
2101 caggtggCGg CGgggCGCGC GcCGggagac cccccctaat ggtgCGtCG ctttctctcaa  
2161 ~~gaggcagttg caagCGCGga ggtgCGaga aataactgcc tcttgaaact tgcagggCGa~~  
2221 atttttagag aaggcaaggc CGgtgtgttt atctgcaagc cattatactt gccaCGaat  
2281 ctttgagaac attataatga cttttgtgcc tcttcttgca agtgttttc tcagctgta  
2341 tctcaagac gatataaa aaactcacca tctagcctta attctcttc ctctacaac  
2401 tgcagtcaat ccatcttacc cctggagcaC Ggtccatat acataccttc ctctatgta  
2461 gacagccacc atgaatatcc agccatgaca ttctatagcc ctgctgtgat gaattacagc  
attcccagca atgtcactaa cttggaagggt gggcc

FIGURE 8B

Unmethylated 288 BP

G ggTGtttttg agatTGtTGg FUM 21 BP AT 60 (SEQ ID NO:85)

TG agttgTGaTG ggttttgg (SEQ ID NO:86)

ccaaaacc CATGCAact CA RUM 20 BP AT 58 (SEQ ID NO:87)

FM 18 BP AT 60 (SEQ ID NO:88)

CGggaaaag taCGtgttCG t (SEQ ID NO:89)

RM 20 BP AT 60 (SEQ ID NO:90)

FIGURE 8C







## SEQUENCING PRIMERS FOR RASSF1A

**External Primers 294 BP**

gggagtttgagtttatttga

**RASSE1 ext. F**

(SEE ID NO: 122)

accocctaaotaccoccto

**RASSF1** ext. R

(SEQ ID NO: 123)

**Internal MSP Methylated 160 BP**

gugggtagc-gggggc  
gcacacatagctagc

**RASSEI FM (2)**

**RASSEI RM**

(SEQ IP NO:124)  
(SEQ IP NO:125)

(Seq ID NO: 125)

**Internal MSP Unmethylated 180 BP**

ggTGTGtattGGttggagTG RASfF1 FUM  
ctacaaacctttaCACAaCA RASfF1 RUM

## MORE

ctacaaccttcaCacaCA RASS1 RUM

**Run**

(JEP ID NO: 127)  
(JEP ID NO: 128)

NO: 128)

**FIGURE 10B**